U.S. DEPARTMENT OF AGRICULTURE
GRAIN INSPECTION, PACKERS AND STOCKYARDS
ADMINISTRATION
FEDERAL GRAIN INSPECTION SERVICE
STOP 3630
WASHINGTON, D.C. 20250-3630

CHAPTER 15

DIAGNOSTIX - EZ-Tox DON TEST KIT

15.1 GENERAL INFORMATION

The EZ-Tox DON test kit is a rapid enzyme immunoassay incorporating ready-to-use liquid reagents approved for quantitative analysis of DON in wheat, barley, malting barley, and corn.

15.2 TESTING AREA

The extraction solution and other materials used in the Diagnostix EZ-Tox DON test kits (part # 600120-DON) do not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table-top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in the handbook to ensure a safe and efficient work environment.

15.3 TEST PREPARATION

a. Allow reagents to reach room temperature prior to running the test (approximately 20 minutes or when reagent bottles do not feel cool to the touch). Dispense an appropriate amount of Reagent A and Reagent B into the provided reagent reservoirs.

b. Turn on the microplate reader and computer. Initiate the EZ-Tox DON Test Software. Allow the microplate reader to warm up to 37 ºC

c. Secure the microplate into the reader's plate holder with well A-1 in the upper left corner. One column of wells on the microplate is used each time the test is run so 1 to 5 samples may be run at a time. A unique numerical identification for each sample is entered into the software.

15.4 EXTRACTION PROCEDURES

a. Place 50 grams of ground sample into a clean container.

b. Add 250 ml of distilled or deionized water and seal/close securely to prevent spillage.

c. Shake vigorously (by hand or mechanically) for three minutes.

d. Transfer with a disposable transfer pipette 1 - 2 ml of the mixture to micro centrifuge tube. Tube should be nearly full. Use a new pipette for each sample.

e. Cap all tubes and place into the mini-centrifuge (all tubes must be suitably counter-balanced)

f. Spin the tubes for a minimum of 60 seconds.

15.5 TEST PROCEDURES

a. Follow the prompts on the computer screen. Assign an 8 well column (1 through 12) for testing.

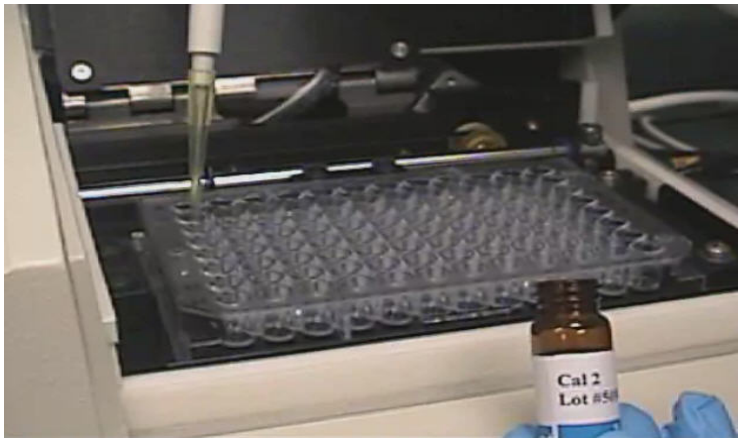
b. With a single channel pipettor:

1. Dispense 25 µl of Cal 1 into the first well (Row A on the microplate).

2. Dispense 25 µl of Cal 2 into the second well (Row B on the microplate).

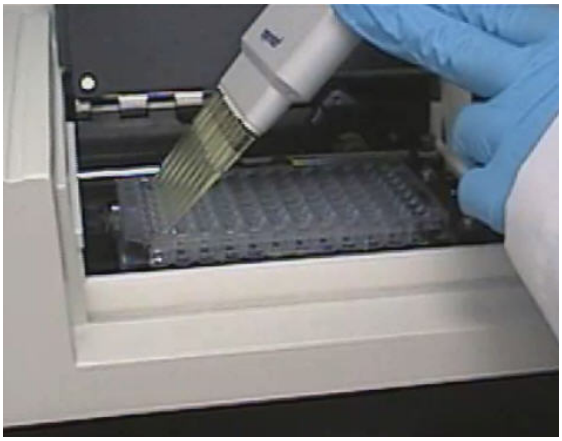
3. Dispense 25 µl of Cal 3 into the third well (Row C on the microplate).

4. Dispense 25 µl of each sample into the next wells (Rows D to H as needed).



NOTE: Use a clean pipette for each addition.

c. With the multi-channel pipettor: Add 100 µl of Reagent A to each well.



d. Close the lid on the reader, check off the completed steps and click the ***SHAKE*** Button.

e. With the multi-channel pipettor: Add 100 of Reagent B to each well.

f. Immediately close the lid on the reader, check off the completed steps, and click on the ***TEST*** button. This is a time sensitive part of the test and the technician needs to be able to add Reagent B and start the testing (by clicking on the Test Button) within 10 seconds.

g. When the run is complete read results directly from the computer screen. Additional options to save or print results are available by following screen prompts.

h. For additional samples go to next available column and repeat steps a through f. Wells cannot be reused.

15.6 QUALITY CONTROL PROCEDURES

The EZ-Tox™ DON Software Program contains an internal quality control function that validates the calibrator values. If these values fall outside the quality control settings, a message is displayed instructing the appropriate course of action.

15.7 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results exceeding 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.

15.8 SUPPLEMENTAL ANALYSIS

If results are above the test method's conformance limit, test results are reported as exceeding the limit. If the applicant wishes to obtain accurate results above the conformance limit, the sample extract must be diluted so that a value **BETWEEN 0.5 AND THE CONFORMANCE LIMIT** is obtained. The final DON concentration is calculated by multiplying the results obtained with the diluted extract by the dilution factor.

For example, if the original analysis reported the DON result at 9.0 ppm and the conformance limit value is 5 ppm, in order to obtain a true value, dilute 5 ml of the original extract with 10 ml of the extraction solution (distilled/ deionized water). The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end). Mix thoroughly and run the diluted extract as a normal sample. Multiply the analytical results obtained by 3 to obtain the actual DON concentration. For example, if 3.1 ppm was the value obtained with the diluted extract, the actual concentration in the original sample was 9.3 ppm (3 x 3.1).

The calculation is as follows:

$$\text{True DON Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{DON Result}$$

In this example: True DON Value = (15 ÷ 5) x 3.1 ppm
= 3 x 3.1 ppm = 9.3 ppm

Laboratories may dilute samples as a first step if levels typically observed in the market exceed the controls provided with the kits.

15.9 CLEANING LABWARE

Clean any reusable labware (e.g., glass collection jars) in a soapy water solution, rinse with clean water, and dry before reusing.

15.10 WASTE DISPOSAL

After the test has been completed, the remaining sample extract and sample solutions may be poured down the drain followed by generous amounts of water. Discard solid material in the trash can for routine disposal.

15.11 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits: EZ-Tox DON test kit

(1) Two 96 well microplates (part #600120-DON) or ten-96 well microplates (part# 600600-DON).

(2) One CAL 1 vial containing 0.10 ppm DON in water.

(3) One CAL 2 vial containing 0.20 ppm DON in water.

(4) One CAL 3 vial containing 0.60 ppm Don in water.

(5) Two Reagent A bottles containing 14 ml of antibody/substrate reagent.

(6) Two Reagent B bottles containing 14 ml of enzyme reagent.

b. Hardware Supplied for test kits: EZ-Tox DON test kit (part number 600120-DON)

(1) Microplate reader.

(2) EZ-Tox Don Software program.

(3) 200 µl multi-channel pipettor.

(4) 100 µl single channel pipettor.

(5) Mini-centrifuge.

(6) Microcentrifuge tube rack.

c. Consumables Supplied by Diagnostix

(1) Microcentrifuge tubes.

(2) Disposable transfer pipettes.

(3) 200 µl pipette tips.

(4) Reagent reservoirs.

d. Materials Required but not supplied by Vendor

(1) Graduated cylinder, 1000 ml.

(2) Sealable containers for sample extraction and extract collection.

(3) Timer.

(4) Wash bottle.

(5) Balance.

(6) Sample Grinder.

15.12 STORAGE CONDITIONS

The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 36° F and 46° F.

On the day the kit will be used it is acceptable to store the kit at ambient temperature (62° F to 82° F).